LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

UCRL-JC-151408

# LLNL Center for Microtechnology: Capabilities, Customers, Case Study-HANAA (Handheld Nucleic Acid Analyzer)

*R. Mariella Jr.*

**December 30, 2002**

Photonics West – January 25-31, 2002, San Jose, California

**UCRL-JC-151408**

# "LLNL Center for Microtechnology: capabilities, customers, case study-HANAA (Handheld Nucleic Acid Analyzer)."

Raymond Mariella Jr.

LLNL

## Abstract

The polymerase chain reaction (PCR) is an enzyme-based chemical reaction that manufactures copies of one or more identifying regions of double-stranded DNA sequences (target sequences)[1-3]. These copies of target DNA are known as "amplicons". By creating millions of these copies of the identifying sequences (when they are present!), PCR allows researchers to detect by them, and hence the presence of the relevant organism, with techniques such as electrophoresis, flow cytometry, or spectrometry. Although there are numerous commercial PCR instruments that are designed for bench-top use in a laboratory, the challenges of building a battery-powered instrument that could perform such assays in the field.

Keywords: instrumentation, polymerase chain reaction, PCR, portable, battery-powered

Recently, Smiths-ETG began commercial production of a hand-held, battery-powered, high-performance PCR instrument, the Bio-Seeq®. (see Figure 1) The following is taken from the web site of Smiths Detection - Edgewood

(formerly Environmental Technologies Group, Inc):

> The Bio-SeeqTM is the first portable, hand-held thermocycler capable of detecting both bacterial and viral pathogens - quickly and accurately using Polymerase Chain Reaction technology. It is highly specific, capable of detecting 1 CFU in as little as 20 minutes. Its small, ruggedized design makes it the perfect hand-held biological agent detector for Homeland Defense and military applications.

The Bio-Seeq® is based on a prototype instrument that we had developed at LLNL, that we called the HANAA (hand-held, advanced nucleic-acid analyzer). The HANAA itself was one of a progeny of instruments that we developed, based on our original invention[4] of a silicon-sleeve-based chamber for thermal cycling.

To perform the selective amplification of DNA via the polymerase chain reaction, which takes place in an aqueous solution, one begins by raising the temperature of the reaction mixture to roughly 96°C, at which temperature each double-stranded DNA, known as the “substrate” DNA, separates into two single strands. One, then, cools the solution to a specific temperature such as 57°C, depending upon the details of the target sequence, which permits reaction the single-stranded DNA primers* to form double-stranded DNA through hydrogen bonds with their complementary DNA sequences on the DNA substrates. The

DNA primers are present in great excess over the concentration of the substrate DNA. The formation of Watson-Crick, double-stranded-DNA pairs[5] between two complementary sequences is referred to as "annealing". After this annealing step, the temperature is typically raised to about 72°C, which is in the middle of the optimal operating temperature range for thermostable (they are not rapidly destroyed by temperature excursions up to 96° C) polymerase enzymes. The primers normally anneal to DNA that is immediately adjacent to the identifying sequence of DNA on the substrate. The polymerase enzyme attaches to the newly-formed, double-stranded DNA of primer with its complementary sequence on the substrate and extends the primer DNA from its 3' end by "reading" ahead on the single-stranded DNA target and covalently attaching one nucleotide at a time from the reaction mixture onto the growing primer. Each nucleotide that is added onto the growing primer is complementary (A with T, G with C) with the next nucleotide on the single-stranded target DNA sequence - the extension process occurs at a rate of approximately 100 bases per second. If the polymerase successfully extends the primers, then the number of molecules of target DNA in the solution has been doubled. Assuming that the reagents have not been damaged or depleted, repeating this process repeats the doubling of the target sequences with every cycle of temperatures. After 30 cycles, the number of identifying sequences of DNA can have been amplified by one billion ($2^{30} \approx 10^9$). Such amplification of target sequences is achieved routinely in laboratory settings with well-engineered instrumentation, running well-developed assays. With some dependence upon the number of copies of the original

substrate DNA, billion-fold amplification of the desired DNA sequence typically produces a sufficient quantity of the desired DNA to detect via gel electrophoresis with non-specific, intercalating fluorophores such as ethidium bromide or Sybr Green®, after the PCR is terminated. While the PCR is running, one does not know how much, if any, identifying DNA sequence is present. One typically decides, a priori, how many temperature cycles one will run (between 30 and 50, normally), and uses electrophoresis only after the total number of cycles has been run. Although the vast majority of PCR that is performed around the world uses such electrophoretic detection of the amplified DNA, the combination of manual sample handling by a technically-trained operator with (typicallyl) the necessity for two laboratory instruments made this approach unappealing for a battery-powered, hand-held PCR instrument.

Therefore, we began designing our PCR instrumentation to utilize the detection of the PCR-amplified DNA via inclusion of fluorogenic probes within the reaction mixture[6]. Specifically, DNA probes with Fluorescence Resonant-Energy-Transfer (FRET) labels, such as Taqman® probes, consisting of a fluorophore and a quencher, have added speed, specificity, and ease of use for assays based upon PCR. The use of fluorogenic probes also greatly simplifies the instrumentation that is needed for the detection of PCR products, since it eliminates the numerous manual steps that are needed in using electrophoretic detection. PCR that uses FRET-based reagents is commonly referred to as “real-time” PCR, because the concentration is monitored during each temperature cycle. This

differentiates the real-time PCR from the more common variety in that one observes the yield of the PCR amplification process as soon as the yield of product reaches the detection threshold, rather than waiting until all the cycles have been run. The number of cycles that one needs to run in order to reach the minimum detection threshold with such a real-time PCR assay is inversely proportional to the starting concentration of target DNA, assuming no cross-reactivity and no inhibitors. As in all PCR assays run on any PCR instrument, the reagents must be validated and reliable[7]. The first battery-powered PCR instrument that LLNL developed, largely supported by ARPA (now called DARPA), used such real-time PCR to detect single-base polymorphisms[8]. Subsequently, with support from the DoD, the LLNL team designed a 10-chamber real-time PCR instrument, called the advanced nucleic-acid analyzer ("ANAA"), based on the silicon-sleeve technology. Although the ANAA performed well[9], there was still an unfilled need for a hand-portable multi-chamber PCR instrument that could run for hours on a small battery. This is what led to the next instrument, called the handheld advanced nucleic acid analyzer (HANAA). HANAA has 4 chambers each with its own optical excitation and detection components for performing real-time PCR, and the new design[10] for the silicon sleeve (see insert in Figure 3) for thermal cycling has significantly decreased the electrical power needed for the process. The typical time for continuous operation of 4 chambers is roughly 2 hours using a single "camcorder"-lead-acid battery. Still, the productivity of the PCR per thermal cycle remained high. Figure 2 shows data collected using the HANAA in January of 2000 starting with

5000 pieces of target DNA in the reaction mixture. The fluorescent signal rose above the baseline at 15 to 16 thermal cycles, which is as good as the performance of other portable instruments, running the same assay with the same reagents. The time for each cycle was slightly less than 20 seconds. A photograph of the HANAA is shown in Figure 3. For a detailed report on HANAA, see reference 11. One lesson that came from the extensive beta testing was that the operating conditions that were required to achieve the high-performance PCR illustrated in the data of Figure 2 were not suitable for general field use. In order to achieve the most rapid PCR, it is necessary to drive the temperature of the silicon sleeve to a set point that is above the boiling point of water for a few seconds and to drive its temperature to a point below the lower set point (below 57° C, for example). Programming the temperature controller to execute such over-driven" conditions for every possible assay was not feasible, at least within the short time frame of commercial development, so that a longer time to perform the temperature cycling is presently utilized in the Bio-Seeq®. Also, the HANAA attempted to use the resistance of the platinum thin-film heater, itself, as the temperature sensor for the control circuitry, and the Bio-Seeq employs a separate temperature-sensing element.

Acknowledgements

This work was performed under the auspices of the U.S. Department of Energy by University of California Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48.

We wish to acknowledge support from ARPA (now, DARPA), the United States Dept. of Defense's Central MASINT Organization, and the United States DOE Office of National Security and Nonproliferation.

References

1. Mullis K; Faloona F; Scharf S; Saiki R; Horn G; Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harbor Symposia on Quantitative Biology*, 1986, **51**:263-73.

2. Mullis K; Faloona F. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in Enzymology*, 1987, **155**:335-50.

3. Saiki R; Gelfand D; Stoffel S; Scharf S; Higuchi R; Horn G; Mullis K; Erlich H. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 1988, **239**:487-91.

4. Northrup MA; Mariella Jr. R; Carrano A; Balch J. Silicon-based sleeve devices for chemical reactions, US Patent #5,589,136 issued 31 Dec 1996.

5. Watson J; Crick F. Molecular Structure of Nucleic Acids: A structure for deoxynucleic acids. *Nature*, 1953, **171**:737-740.

6. Higuchi R; Fockler C; Dollinger G; Watson R. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. *Bio/Technology*, 1993, **11**:1026-1030.

7. Ibrahim MS; Esposito JJ; Jahrling PB; Lofts RS. The potential of 5' nuclease PCR for detecting a single-base polymorphism in Orthopoxvirus. *Molecular and Cellular Probes*, 1997, **11**:143-7.

8. Ibrahim MS; Lofts RS; Jahrling PB; Henchal EA; Weedn VW; Northrup MA; Belgrader P. Real-time microchip PCR for detecting single-base differences in viral and human DNA. *Analytical Chemistry*, 1998, **70**:2013-7.

9. Belgrader P; Benett W; Hadley D; Richards J; Stratton P; Mariella Jr. R; Milanovich F. “PCR analysis of bacteria in 7 minutes”, *Science*, 1999, **284**, 449. The silicon sleeve was 22 mm in height, 6.6 mm wide and 2 mm thick.

10. Benett, W. Patent applied for.

11. Benett W; Richards J; Stratton P; Hadley D; Bodtker B; Nasarabadi S; Milanovich F; Mariella Jr. R; Koopman R; Belgrader P. “Handheld Advanced Nucleic Acid Analyzer (HANAA)”, SPIE International Symposium on Environmental and Industrial Sensing, Boston, MA, 5-8 NOV 2000.

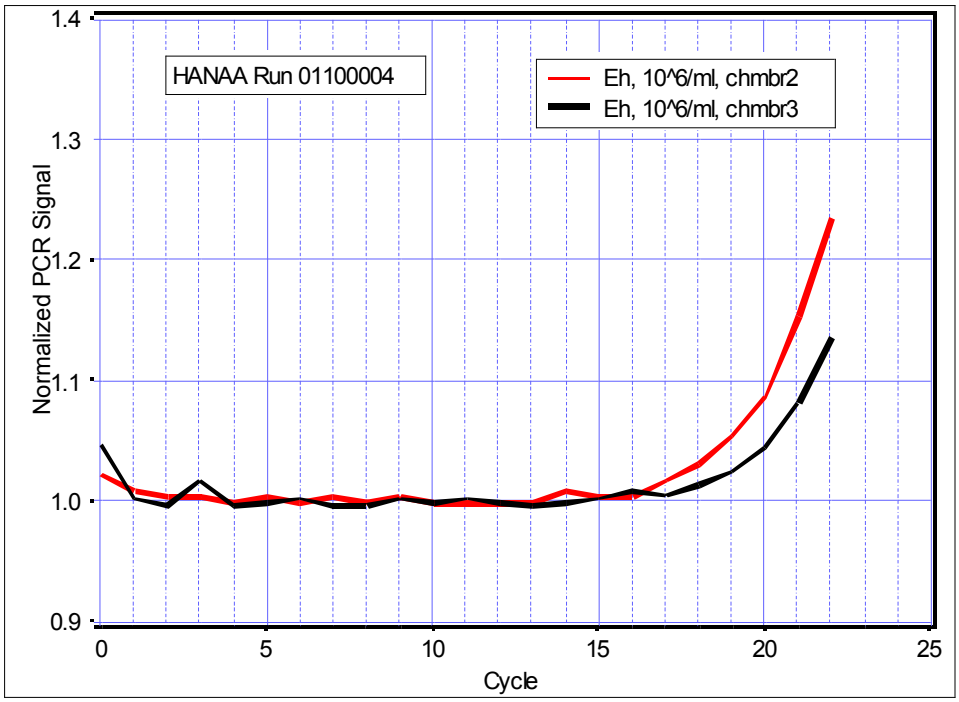
Figure Captions

Figure 1. Photograph of the Bio-Seeq®, courtesy of Smiths Detection, Edgewood.

Figure 2. Data taken using the HANAA in January of 2000, running a PCR assay with Taqman® probe for *E. herbicola*, starting with 5000 bacteria. See text.

Figure 3. Photograph of the HANAA. Insert in upper left is a photograph of latest version of the silicon sleeve design, along with the plastic sample tube.


smiths


HANAA Run 01100004
Eh, 10^6/ml, chmbr2
Eh, 10^6/ml, chmbr3
Normalized PCR Signal
1.4
1.3
1.2
1.1
1.0
0.9
0
5
10
15
20
25
Cycle